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THE NUCLEOLI IN THE SPERMATOCYTES AND GERMINAL VESICLES OF EUSCHISTUS VARIOLARIUS.

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I. In the resting first spermatocyte of many insects there appears a spherical, chromatic body which invariably stains as intensely as the chromosomes.

McClung ('02) was the first investigator to draw special attention to this structure by his suggestion that it may function as a sex determinant.

A majority of the cytologists who have studied this structure interpret it as one of the chromosomes which persists through the rest stage of the first spermatocyte, and they claim that its presence or absence in a spermatozoön is the determining factor of sex, McClung assuming that its presence in a spermatozoön causes the fertilized egg to produce a male, whereas Stevens, Wilson and others affirm that its presence in a spermatozoön causes the fertilized egg to produce a female, and in those cases where two (a large and a small) chromatin nucleoli are present, the larger is the female-determining, and the smaller, the male-determining factor.

Identifying the chromatin nucleolus as a chromosome enlarges at once the sphere of the problem, involving this structure in the maze of hypotheses and theories associated with the chromosomes. This identification of the chromatin nucleolus as a chromosome is of great importance, because it presumably demonstrates that a definite chromosome retains its individuality throughout the rest stage, and it presents very strong evidence for the theory of the individuality and continuity of the chromosomes — the theory which is the corner-stone of all hypotheses involving the

claim that the chromosomes are the cause rather than the expression of cell activities.

We believe that the evidence we find in *Euschistus*, as well as in *Anasa* ('07), is distinctly opposed to the interpretation that the chromatin nucleolus of the resting first spermatocyte is a phase of one or more of the chromosomes, but we shall reserve the publication of this evidence in *Euschistus* until we can control it by a more complete comparison with other forms. In the present paper we shall limit ourselves to the relatively simple question, is the chromatin nucleolus a structure associated with the male cell only, as claimed by McClung, or is it, as claimed by Wilson, a chromosome received from the egg, which during the rest stage appears in the form of a chromatin nucleolus?

Two chromatin nucleoli unequal in size have been demonstrated by Montgomery ('98, '01, '06) in the first spermatocytes of several varieties of *Euschistus*, including *Euschistus variolarius*, and Wilson ('05) has demonstrated these structures in other Hemiptera, naming them chromosome nucleoli, because he believes them to be chromosomes. In describing them in *Cœnus* and *Lygæus* he says: "Throughout the whole of the growth period in *Cœnus*, and from stage *e* onward in *Lygæus*, at least one of the idiochromosomes can always be distinguished as a compact, spheroidal, intensely staining chromosome nucleolus and frequently both idiochromosomes are distinguishable in this form in all of these stages. . . . It is clear beyond all question that at least the large idiochromosome may retain its identity throughout the whole growth period, with the small idiochromosome the case is not so strong" (p. 389). Of *Brochymena* he says: "There can be no doubt that when only one chromosome nucleolus is present it is to be considered as a bivalent body arising by the fusion or synapsis of the two idiochromosomes" (p. 392).

In the resting first spermatocyte of *Euschistus variolarius* the chromatin nucleolus is the most deeply stained and conspicuous structure in the cell (Photos 1–10). As a rule, only one is present, but quite frequently there are two, sometimes equal, but in most cases, very unequal in size; exceptionally we have found three, or even four, nucleoli in the same nucleus.

We roughly estimated the number of the nuclei with one or

more chromatin nucleoli, by counting 625 cells in one testis, and of these cells 591 showed one nucleolus, 27 two nucleoli, and 7 three nucleoli. These 625 cells represented only a small area of the preparation and we did not repeat the experiment on another testis — the estimate, therefore, offers merely an indication that in a large majority of cases only one chromatin nucleolus is present.

By a comparative study of the spermatocytes and oöcytes of the same form we ought to find an answer to the question, whether the chromatin nucleolus is associated with the male cell only, or whether it has its origin in the oöcyte.

As far as we are aware, the germinal vesicles shown in Plates I., II. and III. are the first that have been demonstrated in any of the Hemiptera heteroptera. A great deal has been written about the spermotocytes and important generalizations have been drawn from the behavior of the chromatin nucleolus in these male cells, but not one word has been said about the corresponding, very important stages in the female, although we have been confidently assured that the chromatin nucleolus in the male has been derived from the egg. If this is true, it would seem at least logical to expect to find some evidence of its presence in the egg at the stage of development corresponding to the stage in which it is so conspicuous in the male cell.

Even if we assume for the sake of argument that the chromatin nucleolus is a chromosome, we still do not avoid the logical expectation of finding it in the oöcytes, for if one of the chromosomes of the spermatocyte possesses the distinguishing trait of persisting through the rest stage in the form of a chromosome nucleolus, and if that chromosome is a chromosome contributed to the male cells from the mother, we should expect to see its distinguishing characteristic manifested in even a more marked degree in the oöcyte. And further, as the chromosome nucleolus of the spermatocyte is claimed to be only one of a pair of chromosomes — its mate being contributed by the egg at the time of fertilization — we should expect to find in the resting oöcyte a pair of chromosomes represented by two univalent, or one bivalent chromosome nucleolus.

In the case of *Euschistus* we are told that the larger of the two chromatin nucleoli of the spermatocyte is the homologue of the

accessory chromosome of other forms, and if this interpretation is correct we may expect to find a large bivalent or two univalent chromatin nucleoli in the growing oöcytes.

II. We find the chromatin nucleolus of the spermatocyte persisting through the entire growth period, Photos 1 to 10. All the resting spermatocytes shown in these photos, are from testes which contain many first spermatocyte metaphases, in which the idiochromosomes show the typical inequality in size. We have selected preparations of the resting spermatocytes showing variations in the relative size of the two chromatin nucleoli (when present) in order to demonstrate their frequent lack of conformity to the size relations of the idiochromosomes. Photo 10 shows a nucleolus persisting until the chromosomes are formed and we have several photographs demonstrating its presence to a still later stage, where the seven bivalents can be counted, but we shall reserve the discussion of these later stages for a subsequent paper.[1]

As the chromatin nucleoli of the first spermatocyte of *Euschistus* persist through the entire growth period, we certainly have a right to expect to find in the egg the equivalent of the large chromatin nucleolus during these same stages, if as asserted it owes its origin to the egg. Again, if the so-called male sex-determinant (the small chromatin nucleolus) persists through the growth stages in the male cell, there seems to be no clear reason why the so-called female sex-determinant (the larger chromatin nucleolus) should not persist through the growth period of the oöcyte.

A study of the oöcytes shown in Photos 11 to 30 demonstrates that in none of these cells can be found a structure resembling in any way the chromatin nucleolus shown in the spermatocytes of Photos 1 to 10. This is clearly demonstrated in the spermatocytes and oöcytes of Plate I. There is no structure in the oöcytes of Photos 11 to 19 which resembles in the least the chromatin nucleolus of the spermatocytes of Photos 1 to 10. The only nucleolus in the oöcytes is a relatively large achromatic structure, first clearly demonstrated in the older oöcytes. It is

[1] We have a number of photographs showing the transition stages between Photos 9 and 10, but lack of space prevents their reproduction on these plates.

clearly seen in the germinal vesicles of Photos 18 and 19, Plate I., and in the germinal vesicles of Photos 20 to 25, Plate II., and in Photos 26 to 30, Plate III. All these preparations show, that as a rule its position is peripheral and this is also true of the principal nucleolus of the germinal vesicles of *Allolobophora* (Photo 31). The absence of a chromatin nucleolus is conspicuous not only in the germinal vesicles of *Euschistus* but in the young oöcytes as well. Compare the young oöcytes of Photos 11 and 12 with the young spermatocytes of Photos 1 to 5.

In none of the oöcytes, from the earliest to the latest stages do we find a chromatin nucleolus, and its absence in the germinal vesicles of *Euschistus* is made more conspicuous by the fact that a chromatin nucleolus is invariably present in the germinal vesicles of *Allolobophora*. If we could find such a structure in the germinal vesicles of *Euschistus*, in addition to the achromatic nucleolus, the advocates of the sex determination hypothesis could justly claim it as convincing evidence in support of their theory of its female origin, even if a demonstration of its relation to one of the chromosomes was lacking.

The absence of a chromatic nucleolus in these germinal vesicles cannot be due to the technique since in this case the more delicate achromatic nucleolus would show some disturbance. This point is exemplified in the germinal vesicle of *Allolobophora* (Photo 31), for in this preparation the pricking has disturbed the large, less chromatic nucleolus, while the small, denser, chromatic nucleolus remains perfectly intact, and this condition holds true for the hundreds of germinal vesicles of *Allolobophora* we have preserved.

It therefore seems fair to assume that the technique is not responsible for the absence of a chromatin nucleolus in the germinal vesicles of *Euschistus*.

In an earlier paper [1] we ventured to call attention to the likeness of the chromatin nucleolus in the egg of *Allolobophora* to the chromatin nucleolus of the first spermatocytes of insects. Compare the chromatin nucleolus in *Euschistus*, Photos 1 to 10, with the chromatin nucleolus in the egg of *Allolobophora*, Photos 31 and 32. In both these forms the chromatin nucleolus stains as

[1] Foot and Strobell ('05).

intensely as the chromosomes, and in both forms we sometimes find one and sometimes two chromatin nucleoli.[1] The comparison may be carried even a step further. We may compare the chromatin nucleolus of *Allolobophora* to the chromatin nucleolus of some forms where it is figured at one pole of the first spindle as an undivided chromosome. The chromatin nucleolus of *Allolobophora* often persists through the first division, and in such cases it is either free in the cytoplasm, near the spindle, or at one pole of the spindle in which position it strongly resembles the undivided chromosome figured in so many forms. Photo 32 shows a section through the first maturation spindle of an egg of *Allolobophora*, and a comparison of the chromatin nucleolus at the upper pole of this spindle, with the chromatin nucleolus in the germinal vesicle of Photo 31 leaves no doubt of the identity of the two structures. The identification is further confirmed by the clear demonstration in this and the adjoining sections of all eleven chromosomes in the equatorial plate of the spindle. If the chromosomes of *Allolobophora* were spherical as they appear in sections of so many forms, we could not so easily identify the chromatin nucleolus, for it stains exactly like the chromosomes. Such a case is described by Arnold ('08) in the spermatogenesis of the Coleopteran *Hydrophilus piceus*. During the rest stage the chromatin nucleolus of this form is easily recognized, but he finds it impossible to differentiate it from the chromosomes during the first prophase, because the chromosomes at this stage are also spherical and both structures stain alike. He is able to identify it again at the first metaphase, where it lies in the cytoplasm near the spindle, or within the spindle at one pole. It persists through the first division, but disappears before the second mitosis.[2] It is clear that in its origin, behavior and time of disappearance the chromatin nucleolus of the spermatocytes of *Hydrophilus* closely resembles the chromatin nucleolus of *Allolobophora*, with the exception that the latter does not invariably persist through the first division. Arnold ('08) supports Moore and Robinson's ('05) conclusion that the chromatin nucleolus of

[1] Foot and Strobell ('05), Photo 115.

[2] Wheeler ('97) figured the nucleolus of the germinal vesicle of *Mysostoma glabrum* persisting through the second cleavage and he says that in some cases it persists through the next stage, and perhaps even later, page 35.

the first spermatocyte is not a chromosome and does not take part in either division. His evidence for this is very convincing, he says, if it is to be interpreted as an accessory chromosome which remains undivided in the first spindle it should divide in the second spindle, and half the second spindles should show one more chromosome than the other half. Arnold finds, on the contrary that the number of chromosomes in the second spindle is as constant as the number in the first. He gives in the following table the results he obtained:

Out of 100 counts of first meiotic:

85 per cent. gave.....15 gemini and the nucleolus.
7 " " " over 15 " " " "
8 " " " under 15 " " " "

Out of another 100 counts of first meiotic:

90 per cent. gave.....15 gemini and the nucleolus.
3 " " " over 15 " " " "
7 " " " under 15 " " " "

Out of 100 counts of second meiotic:

91 per cent. gave.....15 chromosomes.
6 " " " over 15 "
3 " " " under 15 "

The resemblance between the chromatin nucleolus of the first spermatocyte of insects and the chromatin nucleolus in the germinal vesicles of some forms has been noted by Häcker ('07) who says, that in those cases in which the heterochromosome is figured as a cap-shaped mass on a large pale plasmosome, the two structures are extraordinarily like the two kinds of nucleoli in the germinal vesicles of the lamellibranch type. He says: "So kann man sich dem Eindruck nicht entziehen, das z. B. das, 'akzessorische Chromosom' in den ruhenden Spermatocyten von Scolopendra (Blackman, 1905, tab. 2, fig. 10–15) früher einfach als 'Nucleolus' beschrieben worden wäre, und man ist geneigt, sich zu fragen, ob denn in allen vorliegenden Angaben bereits eine reinliche Scheidung zwischen den Heterochromosomen und den echten Nucleolen (Plasmosomen) durchgeführt ist. Es sei mir auch gestattet, darauf hinzuweisen, das die von Wilson u. a. gegebenen Bilder, in welchen ein Heterochromosom als

kappenförmige Masse einem grossen Plasmosom aufgelagert erscheint, eine auserordentliche Ähnlichkeit mit den Befunden in den Keimbläschen des Lamellibranchiaten-Typus zeigen, d. h. in denjenigen Keimblässen, in welchen sich zweierlei Nucleolen von verschiedener Lichtbrechung, Quellbarkeit und Tingierbarkeit befinden."

It is a suggestive fact that in *Allolobophora* we have an hermaphrodite form, and if one is determined to regard the chromatin nucleolus of the spermatocytes as a sex-determinant, some interesting conclusions might be drawn from a comparison of the two types of nucleoli observed in this hermaphrodite form, with the two types characteristic of the male and female cells in *Euschistus*. Compare for example the large nucleolus in the germinal vesicle of *Allolobophora* (Photo 31) with the large achromatic nucleolus of the germinal vesicles of *Euschistus* (Photos 18 to 30), and compare further the smaller chromatin nucleolus of *Allolobophora* (Photos 31 and 32) with the chromatin nucleolus of the spermatocytes of *Euschistus* (Photos 1 to 10).

Even if we could be sure that in *Euschistus* the achromatic nucleolus of the egg cells is not represented in the male cells, and that a striking likeness exists between the male and female nucleoli of *Euschistus* and the two types of nucleoli in the germinal vesicles of *Allolobophora*, we would still be unable to claim from the comparison any results of general significance, for the reason that the observations of many investigators of the spermatogenesis of insects offer contradictions to an assumption that the achromatic nucleolus is associated solely with the female cell — these observers having figured a pale, achromatic nucleolus in the spermatocytes, in addition to the chromatic nucleus characteristic of these cells. As opposed to these facts, however, other investigators have found no structure in the spermatocytes that can be interpreted as a nucleolus, other than the characteristic chromatin nucleolus.[1] These conflicting observations, if equally reliable, compel the conclusion that individual forms may differ as to the absence or presence of an achromatic nucleolus.

[1] In his "Studies on Chromosomes — IV.," *Jour. Exp. Zoöl.*, Vol. VI., No. 1, 1909, Wilson in describing *Syromastes*, does not mention a large pale plasmosome, such as he has described in other forms. In *Pyrrochoris* he states that there are from one to three nucleolar-like bodies, which on account of the staining reactions, he believes to be plasmosomes, page 82.

In *Euschistus* we have not been able to demonstrate its presence at any stage of the growth-period of the spermatocytes. In sections we often find faintly staining areas that might be interpreted as an achromatic nucleolus, but in view of the possibility of artefacts in such preparations, we hesitate to interpret them as true nucleoli, unless we can support the interpretation in our smear preparations. Until this point can be settled we are not justified in drawing any conclusions from the obvious difference in type between the nucleoli in the male and female cells of *Euschistus*, though we have here quite as marked a sexual difference of the nucleoli, as any that has been shown for the chromosomes, a difference that appears in no way associated with the maturation divisions of either germ cell.

In comparing the achromatic nucleolus of the oöcytes of *Euschistus* with the principal nucleolus of *Allolobophora* we find some individual differences between the two. The principal nucleolus of *Allolobophora* is clearly differentiated in the young oöcytes as a small, dense chromatic body, and it can be traced uninterruptedly through the entire growth period of the oöcytes. During this period it increases in size, proportionately to the growth of the nucleus, gradually becomes less dense and less chromatic, and finally, when the germinal vesicle has reached its maximum size the principal nucleolus often appears as in Photo 31. In some cases, however, its dense and chromatic character, distinctive of the earlier stages, persists until the chromosomes are fully formed or again it may so completely disintegrate that only a clear space remains as evidence of its existence.[1]

The achromatic nucleolus of *Euschistus* differs from the principal nucleolus of *Allolobophora* in not being present in the young oöcytes as a small dense chromatic nucleolus. We have been unable to demonstrate any such chromatic body in the young oöcytes of *Euschistus*. In the youngest stages in which a nucleolus is found, it appears as clearly achromatic as when it has reached its maximum growth in the germinal vesicle — compare Photos 13 and 16 with Photos 18 to 30. There are also indications that it is often not formed until after the oöcytes have attained a definite growth, for in such clear preparations as are shown in

[1] Foot and Strobell ('05), Photo 122.

Photos 11, 12, 14, 15 and 17, there is no evidence that such a structure is present.[1]

If the nucleolus in the germinal vesicle of *Euschistus* corresponds to the principal nucleolus of *Allolobophora* it must be conceded that there are marked individual differences between the two, differences quite as marked as those existing between the chromosomes of these two forms.

Individuality and Continuity of the Chromosomes.

The investigators who question the individuality and continuity of the chromosomes urge the necessity for further study of the critical stages bearing on this problem — the stages occurring between the end of one mitosis and the beginning of the next. Meves ('08) holds that during these stages it is impossible to recognize the chromosomes. He says: "Man sucht hier, wie O. Hertwig 1890 geschrieben hat, in den Kern etwas hineinzudemonstrieren, was kein unbefangener Beobachter in seiner Struktur erkennen wird.

Fick ('07) claims, that a study of the rest stages proves that the theory of the individuality and continuity of the chromosomes is untenable.

Tellyesniczky ('07) also makes a strong plea for more thorough and careful work on these stages. We are in sympathy with him in his skepticism concerning the individuality and continuity of the chromosomes, but our results differ from his in some, perhaps unimportant, details — differences which may normally exist in different forms. For example Tellyesniczky ('05) believes that a homogeneous distribution of the nuclear substance throughout the nuclear vesicle precedes every mitosis, and the young spermatocytes of our Photos 1, 4 and 5 lend support to this claim. The young oöcyte of Photo 13 also indicates a diffused condition of the chromatin, but as a rule the chromatin of the young germinal vesicles appears as fine granules often very evenly distributed throughout the nucleus as in Photos 11, 14 and 17. Possibly this granular condition may be an artefact — one phase of a precipitate left after drying, for in some cases the

[1] Among recent observations on this point, Deton ('08) finds in *Thysanozoön Brochii* that the nucleolus is absent in the young oöcyte, first appearing at the beginning of the growth period.

chromatin of the young oöcytes is as homogeneous as that of the spermatocytes of Photos 1, 4 and 5.

We find nothing in these germinal vesicles answering to Tellyesniczky's karysomes, unless he would homologize the aggregations of chromatin shown in Photos 12, 15 and 16 with these structures. These aggregations of chromatin are too numerous to be interpreted as the seven bivalent chromosomes of the later stages, or as their fourteen component univalents. In the germinal vesicle of Photo 15, for example, there are about fifty clumps of chromatic substance. Perhaps these clumps of chromatin correspond more closely to the segregated granules Wassilieff ('07) describes in the nucleus of *Blatta*. These clumps, he says, are too numerous to be interpreted as representing individual chromosomes. Later he finds that they disintegrate and are scattered throughout the nucleus like a fine dust; the delicate threads of the later stages being formed at the expense of this dust-like substance.

In the germinal vesicles of *Euschistus variolarius* the chromosomes first appear as extremely fine threads, which seem to arise as a concentration of the distributed granular mass, these granules gradually disappearing as the threads become more defined. Photo 17 shows a germinal vesicle at the stage just before the chromosomes appear — the chromatin is still distributed as granules throughout the nucleus, although the size of this nucleus is nearly equal to many of the germinal vesicles in which the chromatic threads are fully developed. In Photos 18 and 20 we see the delicate chromatic threads just appearing, as yet very indistinct, and in the later stage shown in Photo 21 the threads are more distinct but too much tangled to warrant any precise interpretation. In later stages, however, we can often trace among the filaments, an unbroken thread, so long that it cannot possibly be interpreted as one of the fourteen univalents, it must represent at least two univalents attached end to end. Again some of these threads are long enough to justify the inference that they represent even more than a bivalent, indicating that in *Euschistus* the chromosomes emerge from the rest stage not as univalents, but as long threads representing at least one or more bivalents, thus supporting the observations of those who believe that bival-

ents arise by the omission of a transverse division of a spireme, rather than by a conjugation of univalents. We believe that the evidence in *Euschistus* is opposed to the interpretation that the chromosomes first appear as univalents and later conjugate, either longitudinally or end to end. We believe, rather, that there is a tendency to form a continuous spireme, because we find cases in which very long, thin, unbroken threads are present, and a few examples of this kind outweigh as evidence, a large number of cases where we find the threads broken into small pieces. These delicate threads could easily be broken by the technique, for after pricking, the germinal vesicles flatten and dry on the slide, and this disturbance, combined with the shrinkage of drying, could easily account for the rarity of the cases in which the long thin threads remain unbroken.

In Photo 24 we see this tendency to the formation of a spireme — a few of the threads can be traced certainly too far to justify any assumption that they are univalent or even bivalent chromosomes. A long unbroken thread is also demonstrated in Photos 23, 28 and 29.

Tellyesniczky ('07) claims that the long thin chromatic threads do not become shorter and thicker by contraction. He thinks rather this is accomplished by a flowing together and change of place of the substance of which the thread is composed. He says, there is a continual disintegration and rebuilding of the threads and the new structure is formed at the expense of the old.

We believe this is not the case in *Euschistus*. For example, it seems reasonable to interpret the chromosomes of Photo 30 as due to contraction of long thin bivalents (as shown in Photo 25), rather than to regard them as entirely new formations. Such a detail as this is interesting, but it seems to us only incidental to the all-important question, do the chromosomes retain their individuality throughout the rest stages — do they differ in this particular from other structures of the cell — the centrosome — the aster — the spindle — the nucleolus, etc. These elements return at each cell generation with as much regularity as to size and form as do the chromosomes, but those who believe in the individuality and continuity of the chromosomes attribute to the latter structures a causal significance not now ascribed to any other organ of the cell.

In the past, however, similar claims have been made for the centrosome — its individuality and continuity were asserted by many cytologists, some going so far as to believe the centrosome to be the cause rather than the expression of cell activity.

Certain facts indicate that all the chromatic substance of the germinal vesicle of *Euschistus* is not used for the chromosomes. We find in many germinal vesicles various segregations of chromatic substances which are present after the chromosome threads are formed and which often do not entirely disappear until the prophase stage. We sometimes find irregular granular or homogeneous masses as in Photos 20 and 22, sometimes numerous large granules, so dense that they look like very small nucleoli (Photos 19, 21, 23, 26, 27 and 29), and sometimes merely deeply staining areas, as shown in Photos 23, 24 and 27. We have no means of proving that this chromatic substance, not used for the chromosomes, is chromatin, but for many forms it has been claimed that only a small part of the chromatin is used in the formation of the chromosomes and we are therefore perhaps justified in surmising that the chromatic substance in *Euschistus* (which often persists until after the chromosomes are formed) may be chromatin residue, which assumes various forms during its disintegration and disappearance.

The early stages of the development of the germinal vesicles shown in Photos 11 to 17 we interpret as indicating that the chromosomes completely disintegrate — that any claim of their morphological persistence during these stages must be made as a pure assumption, as there is no morphological evidence whatever of their persistence. On the contrary there is every indication that they completely disintegrate. We believe we have no reason to assume that the reappearance of the chromosomes at certain stages differs essentially from the reappearance of the centrosome, aster, spindle, nucleolus and other cell structures.

Fick ('07) in his critical review of the inconclusive evidence that has been offered as proof of the individuality and continuity of the chromosomes, and of their causal character, sounds a welcome note of protest against recent chromosome speculations. In addition to Fick such experienced cytologists as Häcker and Meves, have expressed their skepticism of the sex-

determination hypothesis in no uncertain terms, and many of the investigators who have studied the accessory chromosomes in insects are equally incredulous, Montgomery, Schäfer, Moore, Robinson, Arnold and others.

We have reserved a description of the later stage of the germinal vesicle chromosomes of *Euschistus* for a subsequent publication, which will discuss only the chromosome groups in both male and female cells. In the male cells we have traced the idiochromosomes through both maturation divisions and are able to support, in the main, Wilson's results as to their division in these stages. He says, the idiochromosomes remain univalent in the first division, each dividing independently, and in the second division the two separate — the small idiochromosome going to one pole and the large idiochromosome to the opposite pole. He gives no details however as to the method of the first division and as we find an interesting irregularity in this division, we believe it is worthy of consideration. As the idiochromosomes separate in the second spindle, this is presumably the so-called reduction division for this unequal tetrad, and the first division should be therefore an equation division, and *both* idiochromosomes should divide longitudinally, if the significance that has been attributed to the longitudinal and transverse divisions of the chromosomes holds true in this form.

The value of the significance of a longitudinal or transverse division is based on the assumption that the so-called ids are arranged in a row, and a morphological demonstration of this assumption is claimed for those cases in which the chromosomes are rod- or thread-shaped — these rods or threads often appearing as a single row of chromomeres.

In our smear preparations of the testes of *Euschistus variolarius* both the large and small idiochromosomes are distinctly rod-shaped, and at the late first prophase and metaphase it can be clearly demonstrated that the *large rod divides longitudinally and the small rod transversely*. There are rare (perhaps only apparent) exceptions to this, but as a rule one divides transversely *just as surely and as constantly* as the other divides longitudinally, and this we have demonstrated in a number of photographs.

The germ cells are being studied in order to obtain a morpho-

logical basis for conclusions or to put conclusions to a morphological test, and the idiochromosomes seem to offer a fruitful test of the theoretical value of longitudinal and transverse divisions. In order to retain our faith in the special significance that has been attributed to the longitudinal and transverse divisions we are driven to the inconsistency of accepting the evidence in the case of one idiochromosome, and disclaiming it for the other — though the evidence in both cases is equally strong. If, on the other hand, we accept the evidence and at the same time retain faith in the theory, we are forced to admit that the sister cells of the first division are also dimorphic and we have a so-called reduction division of one of the idiochromosomes of the first spindle as surely as we have a reduction division (the separation of the two idiochromosomes) in the second spindle, we thus have a somewhat similar phenomenon occurring in both spindles.

We have a number of photographs demonstrating both spermatocyte divisions and also a large number showing the spermatogonial, oögonial and embryonic chromosome groups — several hundred in all, but the publication of these must be reserved for a separate paper, where we shall aim to make a very full comparison of these chromosomes, giving especial attention to the groups that have not been presented in the papers which advocate the sex-determination hypothesis. We believe that these groups are of special value — that we have no right to ignore exceptions because they do not fall in line with certain theories. The trite claim that exceptions prove the rule loses its force when enough exceptions are found to challenge the rule. A careful examintion of our preparations makes it possible to select chromosome groups which exactly fit a given theory, but many groups can also be found that are a serious menace to these theories, while on the other hand they present no difficulties to the conception of those who regard the number, size and form of the chromosomes as inherited characters — the expression of cell activities rather than the cause.

January, 1909.

Appendix.

We have just received Wilson's latest "Study on Chromosomes," No. V.,[1] in which (in a footnote on page 159) he attempts to explain what he calls our "entirely mistaken conclusions" regarding the division of the accessory chromosome in *Anasa tristis* as follows:

"The regrouping of the chromosomes in the second division, first described by Paulmier ('99) in *Anasa tristis*, is characteristic of the Coreidæ generally, an eccentric position of the idiochromosome being a nearly constant feature of the first division but not of the second. Failure to recognize this fact in the case of *Anasa tristis* seems to have been one of the main sources of error in the entirely mistaken conclusions of Foot and Strobell ('07*a*, '07*b*) regarding this species. (Cf. Lefevre and McGill, '08.) Demonstrative evidence on this point is given by polar views of rather late anaphases, in which every chromosome of each daughter plate may be seen, in the same section. Such views, of which I have studied many, both in *Anasa* and other genera, show that one of the chromosomes may indeed occupy an eccentric position, and may there divide; but in such cases the odd chromosome is always found elsewhere in the group, lying either in or near one of the daughter groups and not in the other. When the odd chromosome is eccentric it is found in one of the daughter groups but not in the other."

In order to speak with authority on the regrouping of the chromosomes, some accuracy at least is expected in the identification of the individual chromosomes. Paulmier's authority can be dismissed when we recall Wilson's interesting discovery that in *Anasa tristis* Paulmier erroneously identified the microchromosome as the accessory (undivided) chromosome of the second spindle.

In Plate III. of our "Study of Chromosomes in the Spermatogenesis of *Anasa tristis*" ('07) we *show* in Photos 27, 28, 33, 35, 36, 37, 38, 39, 42, 43, 44 and 45, late anaphases or early telophases of second spindles demonstrating the ring-like formation of the chromosomes, with one of the chromosomes eccentrically placed or lagging. In our smear preparations this grouping is

characteristic of the second division as it is of the first, although, as we have already pointed out, there are exceptions to this rule. There can be no possible doubt of our identification of these groups as second spindles, because they are invariably found in regions of the testis surrounded by spermatids in different stages of development and, further, the form of the chromosomes is so beautifully demonstrated in our smear preparations, it is hardly possible to confound the first and second spindles,[1] a mistake easily made in sections. That the individual chromosomes may change their relative positions at this stage, while the form of the group remains unchanged, is a possibility that any observer would naturally consider. But as we find in our preparations, the ring-like grouping of the chromosomes of the first spindle so constantly duplicated in the second spindle, with the micro-chromosome (the one chromosome that can be identified beyond question) maintaining its characteristic position in the center of the ring, the identification of the eccentric chromosome of both spindles as *probably* the same chromosome, is certainly as legitimate a conclusion, as forcing an arbitrary contradiction of this assumption, in order to support a theory.

In complete opposition to what Wilson calls his "demonstrative evidence" on this point, we refer to our published photographs, Plate III. (in the paper quoted above), 29, 32, 33, 34, 35, 36, 37, 40, 41, 43, showing polar views of late anaphases or telophases of second spindles, in which every chromosome of each daughter plate can be counted. In all these preparations there is an eccentric, lagging — in most cases dyad — chromosome, but as *ten* chromosomes can be clearly counted at each pole, we fail to see how the situation is improved, for Wilson to claim, that where the lagging chromosome divides in this spindle it is not to be identified with the "odd" chromosome — which he says, "is, in such cases, *always to be found elsewhere in the group.*" We have given demonstrative evidence that this is not so in our preparations.

The essential fact is not the relative position of the accessory chromosome in either the first or second spindles, but whether

[1] The ring-like grouping of the chromosomes is also characteristic of the first and second spindles of *Euschistus variolarius*, where the distribution of the unequal tetrad in the second spindle adds conclusive proof of the identity of the spindles.

one of the chromosomes consistently fails to divide in the second spindle. It has been said of the accessory chromosome that in the spindle in which it is heterotropic, it tries to divide, but does not succeed. In the case of *Anasa tristis*, we have demonstrated that it is by no means always unsuccessful in these efforts.

We regret that we cannot agree with Professor Wilson as to the value of the support he believes he has received from Lefevre and McGill's paper on *Anasa tristis* and *Anax junius* ('08). In this paper our results in *Anasa* are summarily disposed of in two pages of curt contradictions, supported by ten sketches made from sections. The futility of such methods as opposed to the very full demonstration we have given in 107 photographs to support our conclusions in *Anasa*, must be apparent to any unprejudiced observer.

In this same paper, McGill corrects her earlier observations on *Anax junius* on three very important points :

1. In her first work on this form ('04) (in which she expresses her indebtedness to Professor Lefevre) she found no evidence of a chromatin nucleolus in the resting spermatocyte. She and Lefevre now find, *in the same material*, a chromatin nucleolus persisting through the resting spermatocyte.

2. In her earlier work McGill identified the microchromosome as the accessory chromosome. She and Lefevre now identify, *in the same material*, one of the larger spermatogonial chromosomes as the accessory chromosome.

3. In her original count of the spermatogonial chromosomes, McGill found an even number, 28. She and Lefevre now find *in the same material* an odd number, 27 spermatogonial chromosomes.

In view of these contradictions we may justly hesitate to accept as definitive the recent conclusions reached by McGill and Lefevre in *Anasa tristis*, believing a new point of view may give us still further variations in their very interesting observations.

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Explanation of Plates.

All the photographs were taken with a Zeiss Apo. 2 mm. immers. lens, 140 apr. and compensating ocular 4. Photos 1 to 31 are from smear preparations stained with a saturate solution of Bismarck brown. In all these preparations the *whole nucleus* is shown.

Magnification of Photos 1 to 10 inclusive, 1,000 diameters.

Magnification of Photos 11 to 32 inclusive, 600 diameters.

As some of the germinal vesicles were too large to be included in the field at a magnification of 1,000 diameters, we used a magnification of 600 diameters for all the oöcytes, in order to facilitate a comparison.

The reproductions were made by the Rotograph Company from our own negatives.

Plate I.

Euschistus variolarius.

Photo 1. Two young resting spermatocytes, first order, one showing two nucleoli and the other one nucleolus.

Photo 2. A little later stage than Photo 1. Two nucleoli are shown, unequal in size, but this inequality is not so marked as in the two nucleoli of Photo 6.

Photo 3. Resting first spermatocyte about the same stage of development as Photo 2. One nucleolus present.

Photo 4. A resting first spermatocyte showing two nucleoli about equal in size.

Photo 5. A resting first spermatocyte with two nucleoli, unequal in size, though the inequality here is not so conspicuous as in Photo 6.

Photo 6. A later stage than Photo 5. The chromatin shows the segregation which precedes the formation of the chromosomes. Two nucleoli are present, very unequal in size.

Photo 7. First spermatocyte with the chromatin somewhat more closely segregated than in Photo 6. One nucleolus is present showing a typical vacuole.

Photo 8. A larger first spermatocyte with one nucleolus, the disposition of the chromatin suggesting a network.

Photo 9. A first spermatocyte showing an early stage in the formation of the chromosomes; one nucleolus is present.

Photo 10. A much later stage than Photo 9. The bivalent chromosomes are formed, but the number is not so clearly demonstrated as at later stages. (These later stages, as well as the transitional stages, between Photos 9 and 10, are reserved for a subsequent publication.) The nucleolar-like thickening in the loop of the chromosome on the lower periphery of the group is not a small nucleolus. In the preparation it can be plainly recognized as a segregation of chromatin at the point where a loop of the chromosome brings two parts of the thread in contact. Similar, though less conspicuous thickenings are seen at other points where two threads cross. One clear nucleolus is present in this spermatocyte.

Photo 11. Nucleus of a young oöcyte, with the chromatin granules quite evenly distributed throughout the nucleus. No nucleolus present.

Photo 12. A slightly older germinal vesicle, with the chromatin segregated into clumps, too numerous to represent individuual chromosomes.

Euschistus variolarius.

FOOT & STROBELL PHOTOS.

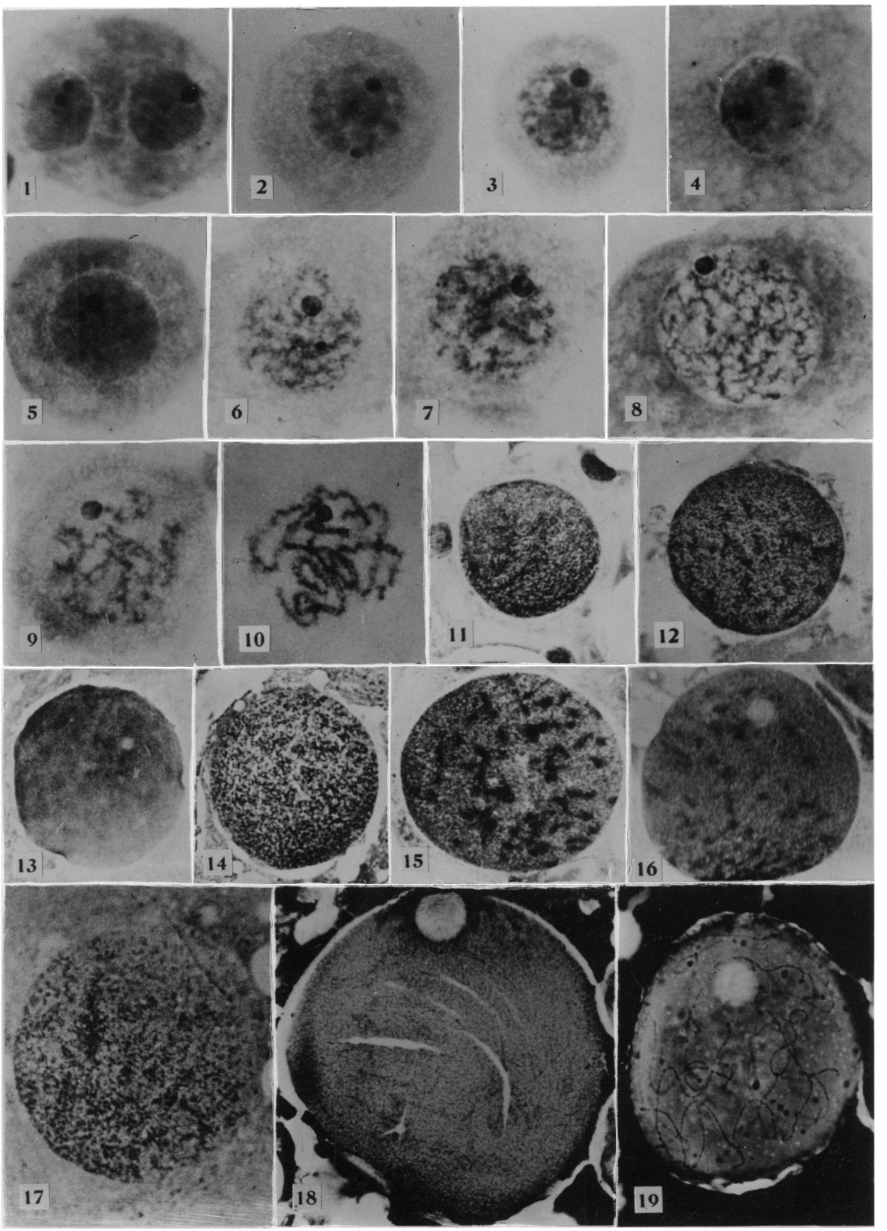

PHOTO 13. A young germinal vesicle with the chromatin almost in a diffused condition. A small, clear, spherical space is present, which may represent the first appearance of the achromatic nucleolus so conspicuous in the older germinal vesicles of Photos 18 to 30.

PHOTO 14. A young germinal vesicle with the chromatin evenly distributed as in Photo 11.

PHOTO 15. An older germinal vesicle with the chromatin segregated into numerous clumps as in Photo 12. Many of the clumps in this germinal vesicle appear almost as dense as nucleoli, though in the preparation they are seen to be merely a close segregation of fine granules.

PHOTO 16. An older germinal vesicle showing less pronounced segregations of chromatin, and a clear spherical space which can surely be interpreted as an early appearance of the achromatic nucleolus seen in the germinal vesicles of Photos 18 to 30.

PHOTO 17. A much larger germinal vesicle with the chromatin quite evenly distributed as granules.

PHOTO 18. An older germinal vesicle with the first indication of the formation of delicate chromatic threads, which later develop into the chromosomes. At this stage the achromatic nucleolus is always present. The cracked spaces shown in this germinal vesicle and in Photos 21, 23, 24 and 29 are due to uneven shrinkage as the germinal vesicles rapidly dry on the slide.

PHOTO 19. A much later stage than Photo 18 — the chromatin threads being well formed. (The next stage to Photo 18 is shown in Photo 20, Plate II.) The germinal vesicle of this preparation is not well flattened and some of the chromatic threads are very much out of focus.

PLATE II.

Euschistus variolarius.

PHOTO 20. A germinal vesicle a little further developed than the one shown in Photo 18. The delicate chromatic threads are a little more defined. The achromatic nucleolus is present near the periphery.

PHOTO 21. A germinal vesicle with the delicate chromatic threads well formed. The achromatic nucleolus is seen on the periphery.

PHOTO 22. A germinal vesicle with the chromosome threads further developed. The nucleolus near periphery.

PHOTO 23. A germinal vesicle with long, thin chromosome threads. The nucleolus on periphery.

PHOTO 24. A germinal vesicle at about the same stage of development as Photo 23 The nucleolus on periphery.

PHOTO 25. A germinal vesicle with the chromosome threads further developed.

FOOT & STROBELL PHOTOS.

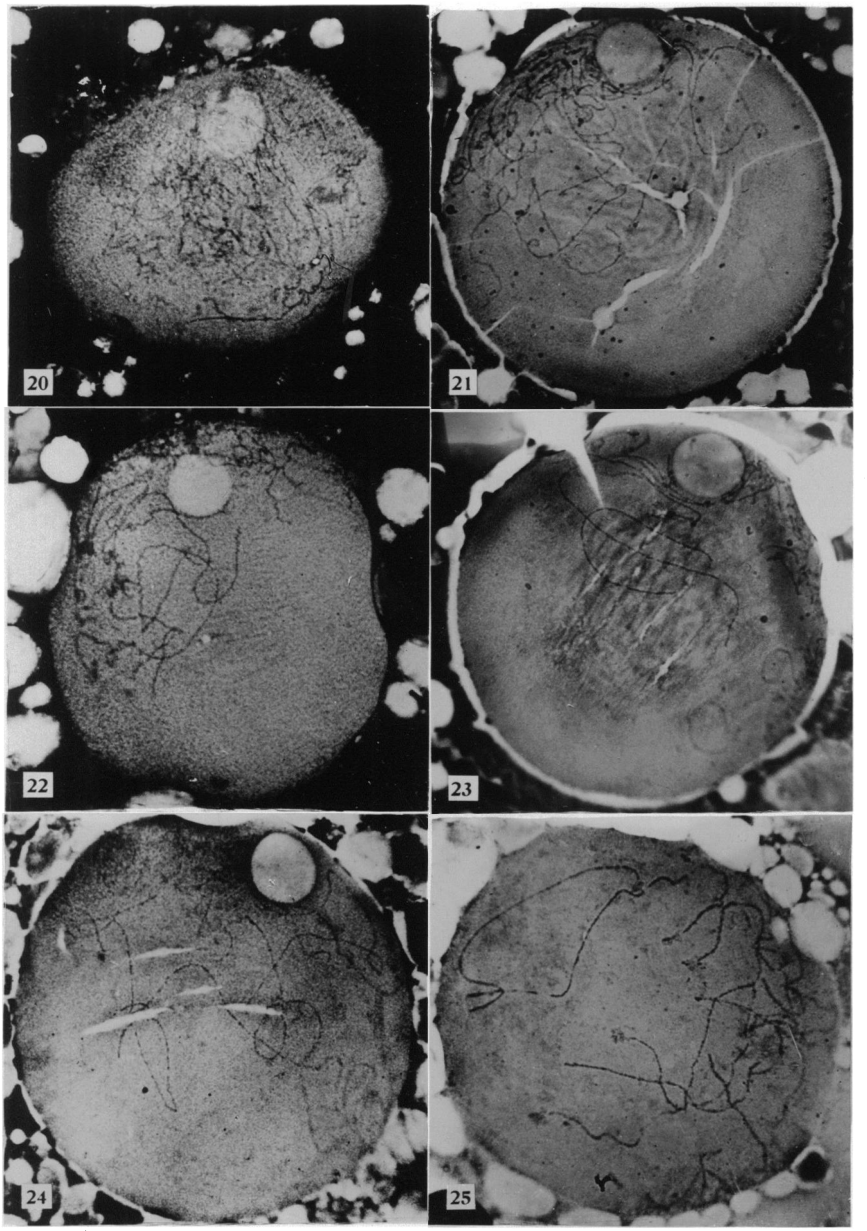

PLATE III.

Euschistus variolarius and Allolobophora fœtida.

PHOTO 26. A germinal vesicle with chromosome threads well formed. Nucleolus on periphery.

PHOTO 27. A germinal vesicle at about the same stage of development as Photo 26. The nucleolus near the center.

PHOTO 28. A germinal vesicle with long, thin, twisted chromosome threads. Nucleolus near the center.

PHOTO 29. A germinal vesicle showing a later stage of development. The chromosome threads have contracted into shorter, thicker pieces. Nucleolus still present.

PHOTO 30. A germinal vesicle with the chromosomes almost at first prophase stage. Nucleolus still persisting.

PHOTO 31. A germinal vesicle of *Allolobophora fœtida*. The same technique as that used for the *Euschistus* germinal vesicles (Photos 18 to 30). The chromosome threads are long, and thin, and longitudinally split. The large, less chromatic nucleolus is on the periphery, and the dense chromatin nucleolus is near the center.

PHOTO 32. A section (2½ μ thick) through the first maturation spindle of *Allolobophora fœtida*. At the equator one chromosome is shown, the ten remaining chromosomes being clearly demonstrated in the adjoining sections. Near the upper pole we see the persisting chromatin nucleolus. A centrosome is shown at each pole

Euschistus variolarius.

FOOT & STROBELL PHOTOS.